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Photo-Signal Transduction in Motile Cilia <u>Blepharisma</u>						03-92-G0356
6. AUTHOR(S)		***				
Pill-Soon Song						
7. PERFORMING ORGANIZATION NAMES(S) AND ADDRESS(ES)					8. PE	RFORMING ORGANIZATION
The Board of Regents of the Univ. of NE-Lincoln						PORT NUMBER
303 Canfield Administration Building Lincoln, NE 68588-0430					T.WF	7/05-139-10402
HIROTH, NE 08388-0430					2.01	703-139-10402
9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)					10 85	PONSORING / MONITORING
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U.S. Army Research Office P.O. Box 12211						
Research Triangle Park, NC 27709-2211					0.0	0 29597.4-LS-EPS
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11. SUPPLEMENTARY NOTES						
The views, opinions and/o	r findin	gs contained in th	is report ar	e those of the auth	or(s)	and should not be construed as
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the chemical structures of both blepharismin and stentorin. How these light sensor molecules mediate the intensity- and wavelength-sensitive light-sensory responses in these single cell organisms is still under						
investigation in this laboratory, results so far strongly suggest that both blepharismin and stentorin initiate their						
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14. SUBJECT TERMS						
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Light-signal transduction, photosensor molecules, protozoan ciliates, Blepharisma japonicum, Stentor coeruleus						4
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17. SECURITY CLASSIFICATION	18. SEC	URITY CLASSIFICATI	ON 19. SE	CURITY CLASSIFICA	TION	20. LIMITATION OF ABSTRACT
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Photo-Signal Transduction in Motile Cilia <u>Blepharisma</u>

FINAL PROGRESS REPORT

Pill-Soon Song

U.S. ARMY RESEARCH OFFICE

Funding Number: DAAL03-92-G-0356

University of Nebraska-Lincoln Department of Chemistry

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REPORT DOCUMENTATION PAGE (SF298) (Continuation Sheet)

Specific aims of the study were to elucidate: (a) the chemical structures of light-sensor molecules blepharismin and stentorin, and (b) their mechanisms of action in light-sensory signal processing events, namely, detection of sudden changes in light intensity and light-avoiding behaviors of the single cell ciliate Blempharisma japonicum and its closely related organism Stentor coeruleus.

During the period of this grant, two significant results have been achieved. For the first time, we have been able to isolate and purify the photosensor-bound protein, stentorin, with molecular weight of 55,000. This light-sensor protein retains the functional characteristics of native stentorin protein complex of nore than half a million molecular weight located within the pigment granules of *Stentor coeruleus*. In addition, we have been able to determine a tentative chemical structure for the light sensor chromophore of *Blepharisma japonicum*. It appears that the chemical structure of blepharismin is distinctly different from that of stentorin. Both stentorin and blepharismin add to the exclusive list of only a limited number (four to five) of light sensor molecules in nature.

Light signals perceived by the single cell ciliates are transduced by transducin-like G-proteins, as suggested by our study of the effects of various G-protein activators and inhibitors on the photo-sensory responses of both organisms. To characterize the G-proteins in the ciliate cells, Phun Bum Park and Elisabetta Bini supported by this grant partially cloned and sequenced Blepharisma and Stentor G-proteins. Photo-activation of G-proteins appears to be coupled to a cGMP-dependent phosphodiesterase. If this result is further confirmed by directly isolating and/or cloning the latter and establishing its light activation via G-proteins, the single cell photo-signal transduction system will provide an interesting comparison to the visual excitation system in mammals.

- (1) LIST OF MANUSCRIPTS published under ARO sponsorship during this grant period (includes publications arising from both DAAL03-92-G-0356 and 28748-LS-SM with identical project title):
- 1. N. Tao, M. Orlando, J.-S. Hyon, M. Gross and Pill-Soon Song, A New Photoreceptor Molecule from *Stentor coeruleus*. J. Am. Chem. Soc. 115, 2526-2528 (1993).
- 2. **Pill-Soon Song**, Structure and Function of the Ciliate Photoreceptors. In: <u>Frontiers of Photobiology</u> (Edited by A. Shima, M. Ichihashi, Y. Fujiwara and H. Takebe), International Congress Series 1021. Excerpta Medica, Amsterdam and New York, 1993. pp. 153-157.
- I. Yamazaki, T. Yamazaki, Y. Nishimura, R. Dai and Pill-Soon Song, Time-Resolved Fluorescence Spectroscopy and Photolysis of the Photoreceptor Blepharismin. <u>Biochim. Biophys. Acta</u> <u>1143</u>, 319-326 (1993).
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- 5. S. Fabczak, H. Fabczak, N. Tao and **Pill-Soon Song**, Photosensory Transduction in Ciliates. I. An Electrophysiological Analysis of the Photophobic Response in *Stentor coeruleus*. <u>Photochem. Photobiol.</u>, **57**, 696-701 (1993).

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- 12. N. Tao and **Pill-Soon Song**, New Light Sensor Molecules of Single-Cell Ciliates. <u>Smart Structure</u> and Materials: SPIE Proceedings, Vol. <u>2189</u>, 238-248 (1994)
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